F105 The analysis of the 5' non-coding region and PCR/RFLP-based identification of enteroviruses isolated from patients with aseptic meningitis in Korea

Yoon Sung Lee[1], Yoon Seok Chung[1,2], Ki Soon Kim[1,2], Jeong Koo Park[2], Ji Eun Kim[2], Young Mee Jee[2], Moon Bo Kim[1], Dong Soo Kim[3], Jae Deuk Yoon[2], Chul Yong Song[1], and Kwang Ho Lee[1]

Dept. of Life Science, College of Natural Science, Chung-Ang Univ. [1], Lab. of Enteroviruses, Dept. of Virology, NIH[2], Dept. of Pediatrics, Yonsei Univ. College of Medicine[3]

A current diagnostic procedure for isolation and typing of enteroviruses involves the cell culture using neutralizing antisera, which is laborious and not sensitive enough to distinguish several specific serotypes. Therefore, this study has focused on the development of an alternative method for the rapid detection and identification of enteroviruses causing aseptic meningitis. The 5' non-coding regions (NCRs) of seven serotypes of enteroviruses, coxsackieviruses B1, 2, 3, and 5, and echoviruses 6, 7, 9 and 30, which isolated from patients with aseptic meningitis in Korea since 1993 and previously typed by using Rijksinstituut voor volksgezonheid (RIVM) serum pools, were analyzed. The multiplex PCR analysis was performed with the primer sets derived from P1 and P2 regions of polioviruses to exclude poliovirus from non-polio enteroviruses. The internal sequence of the 5' NCR of each enterovirus isolate, which is known to be highly conserved among different serotypes, was also amplified by reverse transcription (RT) - polymerase chain reaction (PCR) followed by direct sequencing. Based on the sequence analysis, the 5' NCR of the enteroviruses was found to have several different restriction enzyme recognition sites depending on their serotypes, and showed restriction fragment length polymorphisms (RFLPs). With these different RFLP patterns of the PCR products, we could differentiate 8 different serotypes of enteroviral isolates. In blind examinations of about 100 clinical isolates, the RFLP-based typing exactly corresponded to the results of conventional serotyping by RIVM serum pools. Our results indicate that PCR-RFLP of the 5' NCR of enteroviruses allows the rapid diagnosis and subtyping of enteroviruses.

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F106 Cloning and expression of a putative group-common epitope of enteroviruses

Yoon Sung Lee[1], Yoon Seok Chung[1,2], Ki Soon Kim[1,2], Jeong Koo Park[2], Ji Eun Kim[2], Young Mee Jee[2], Moon Bo Kim[1], Dong Soo Kim[3], Jae Deuk Yoon[2], Chul Yong Song[1], and Kwang Ho Lee[1]

Dept. of Life Science, College of Natural Science, Chung-Ang Univ. [1], Lab. of Enteroviruses, Dept. of Virology, NIH[2], Dept. of Pediatrics, Yonsei Univ. College of Medicine[3]

Since there are over 60 different kinds of enterovirus serotypes, cloning of a group-common epitope of enteroviruses would play a critical role for developing enteroviral vaccine candidates and immunological diagnostic tools. This study has focused on the cloning of a group-common epitope of enteroviruses. To clone this cross-reactive antigen among different serotypes of enteroviruses, cDNA libraries of Korean isolates of E7, E30, and CB3 have been constructed with λ gt11 arms. By using an immunoscreening technique with the convalescent sera from the patients with aseptic meningitis, the cDNA clones with the high immuno-reactivity were selected and analyzed. Total 61 of 81 positive (immuno-reactive) plaques (clones) were characterized by a PCR-direct sequencing method, and the genomic position of each individual clone was determined by comparing nucleotide sequences of the positive clones with nucleotide sequences of enteroviruses, which had been published or accessed in the GenBank database. Based on the sequence analysis, over 50% of the immuno-reactive cDNA clones were found concentrated on the N-terminus of VP1 and the C-terminus of VP3. Therefore, we have amplified this region of E7 with a primer sets E7ys1F and E7ys1R which were designed by analysis of nucleotide sequences above, and have cloned into pET30a to express it in the prokaryotic system. Antigenicity of the expressed proteins was analyzed by western blot analysis using anti-E7, E30, CB2, CB3 and CB5 guinea pig polyclonal antibodies. All the polyclonal antibodies used in this study showed the strong immuno-reactivity with the putative common antigen. Our results indicate that the E7 cDNA clone is a common major epitope among different serotypes of enteroviruses and the protein expressed could be used as a potential antigen for the serological diagnostic system. [Supported by grant from MOHW, No. HMP-96-D-6-1054]